EXHIBIT F

TO DECLARATION OF SCOTT D. TANNER, PHD.

U.S. Patent Application Ser. No. 10/614,115

RECEIVED AUG - 4 2005

DEPARTMENT OF HEALTH & HUMAN SERVICES — Public Health Service

National Institutes of Health
National Institute of
General Medical Sciences
Bethesda, Maryland 20892-620(
http://www.nigms.nih.gov

JUL 2 5 2005

Mitchell A. Winnik, Ph.D.
University of Toronto
Dept of Chemistry
80 St George St
Toronto Ontario Canada M5S 3H6,

C3HCE

Re: 1 R01 GM076127-01 ~~C000CE~~ OCT 2005 Council

Dear Dr. Winnik:

I am enclosing a copy of the summary statement prepared by the scientific review administrator of the initial review group (IRG) that evaluated your application. Also enclosed is an information sheet (NIGMS Staff Actions on Applications after Initial Review) explaining the actions that NIGMS staff may take with regard to applications. The actions or rank that may be recommended by an IRG are underlined. Please consult this sheet to ascertain which NIGMS staff action pertains to your application. If your application received a rating that makes it unlikely for funding, please be assured that this will in no way prejudice the independent consideration of any applications (revised or new) that you may submit in the future. The third enclosure describes the appeal process.

REMINDER for Required, Countersigned Information: If your application, with the exception of T32 or R25 applications, received a percentile ranking between 0.1 and 20.0, or if a percentile ranking is not specified and the priority score is between 100 and 250, please submit Other Support information within two weeks of receipt of the Summary Statement. Further, if your research involves human subjects, you must submit information on required education in the protection of human research participants for all key personnel. Please refer to the enclosed NIGMS Staff Actions sheet for instructions.

This letter is not intended to imply anything about the ultimate funding status of your application. If you have questions about the peer review process or concerns about the scientific review of your application, I encourage you to discuss them with me, your program director, as soon as possible. For information about the business aspects of your application, you may contact the grants management officer identified below.

Sincerely yours,

Charles G. Edmonds, Ph.D.
Program Director
Cell Biology & Biophysics Division
Phone: (301) 594-0828
E-Mail: EDMONDSC@NIGMS.NIH.GOV

The Grants Management Officer is:
Mr. Joseph Ellis
Phone: (301) 594-5135
Fax: (301) 451-5601
E-Mail: ELLISJ@NIGMS.NIH.GOV

Enclosures (3)
Business Official (Letter only)

SUMMARY STATEMENT
(Privileged Communication)

Charles Edmonds
(301) 594-0828
edmondsc@nigms.nih.gov

Release Date: 07/22/2005

Application Number: 1 R01 GM076127-01

WINNIK, MITCHELL A PHD
UNIVERSITY OF TORONTO
DEPT OF CHEMISTRY
80 ST GEORGE ST
TORONTO, ONTARIO M5s 3H6

Review Group: EBT
Enabling Bioanalytical and Biophysical Technologies Study Section

Meeting Date: 06/23/2005
Council: OCT 2005
Requested Start: 12/01/2005

RFA/PA: PA05-001
PCC: C312CE

Project Title: Metal tagging of bioactive molecules for prognostic assays coupled with ICP-MS

SRG Action: Priority Score: 229 Percentile: 32.5
Human Subjects: 10-No human subjects involved
Animal Subjects: 10-No live vertebrate animals involved for competing appl.

Project Year	Direct Costs Requested	Estimated Total Cost
1	225,000	243,000
2	225,000	243,000
3	225,000	243,000
4	225,000	243,000
TOTAL	900,000	972,000

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

BUDGET MODIFICATIONS FOREIGN NEW INVESTIGATOR

1R01GM076127-01 WINNIK, MITCHELL

FOREIGN INSTITUTION
NEW INVESTIGATOR
COMMITTEE BUDGET RECOMMENDATIONS

RESUME AND SUMMARY OF DISCUSSION: This application from a new investigator proposes the development of a new method for metal tagging antibodies to achieve a very sensitive bioanalytical assay tool using inductively-coupled plasma mass spectrometry (ICP-MS) for detection. There was an initial difference of opinion among panel members about the overall merit of this application. Strengths noted were the highly innovative aspects of the approach, the potential impact of being able to achieve multiplexed analyses, and the outstanding and complementary expertise of the investigative team. However, some weaknesses were also mentioned. The advantage of using chelating polymer tags rather than multiple metal nanoparticles was not clear, and the possibility of nonspecific binding at the unchelated sites was a concern. In addition, the biological problem driving the development of this tool was not apparent. Following the discussion, the difference of opinion narrowed considerably, and a majority of the reviewers expressed a moderate level of enthusiasm for this application.

DESCRIPTION (provided by applicant): The objective of this proposal is development of an advanced metal tagging systems and methodology, which in combination with an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) detector will provide researchers and clinicians with substantially improved analytical and prognostic capabilities. ICP-MS as an analytical detector possesses decisive advantages that enhance the performance of immunoassays, including: i) high precision; ii) low detection limits; iii) large dynamic range; iv) independence of non-specific background and analytical response from incubation or storage times (as protein degradation does not affect analysis of an elemental tag); v) larger multiplexing potential (potentially, up to 167 isotopes; realistically, around 100 distinguishable tags. Mismatch of excitation and absorption wavelengths, and overlap of emission signals are physical characteristics of fluorophores (including quantum dots), which represent a critical barrier to progress in the field of multiplexed assays and cannot be eliminated. A key feature of our approach is the development of tagged affinity products designed for specific recognition of distinguishing cell surface and intracellular markers and targeted for the ICP-MS detection. Methods of labeling with distinguishable stable isotopic elemental tags will be developed. This project will deliver stable metal tags, appropriately tagged affinity reagents, methods and demonstrative data for the distinction of multiple analytes. We hope that this integrated reagent system will dramatically enhance the diagnostic, prognostic and therapeutic efficacy available to physicians and their patients, increasing the effectiveness of healthcare while substantially reducing the human and financial costs of modern personalized treatment. Our interdisciplinary group of recognized experts will address this significant challenge. Professors Mitchell A. Winnik and Mark Nitz will use existing capabilities to synthesize the carrier polymers. Methods of tagging with distinguishable stable isotopic elemental tags will be developed. The ICP-MS group (Vladimir I. Baranov) will develop a purpose specific analytical methods combining robotic sample introduction with an ICP-MS instrument that will allow multiplex detection. Development of the elemental tagged assay methodology will be conducted by Olga I. Omatsky using existing capabilities and in constant verification against conventional assay methods.

CRITIQUE 1:
Significance: It would be highly advantageous to perform immunoassays in a multiplexed fashion, where a complex mixture of selective antibodies can identify, for example, an unknown pathogen, rather than testing the pathogen with many different antibodies. It could save labor, expense and sample. If this multiplexing could achieved with detection limits that are comparable to fluorescence, this would be extremely valuable. These are the essential ideas behind the proposal, and a credible case is made for this being achievable. Metal tags provide the multiplexing capability by virtue of giving unique combinations of emission spectra and mass spectra. The proposed research could potentially have an enormous impact on immunoassays, bring down the cost and speed of medical testing, and likely saving lives by identifying pathogens faster.

Approach: What is proposed to be done is to develop a means of attaching multiple metal atoms to antibodies, choosing commonly used systems as models suitable for developing the methodology. The sensitivity relies upon having many metal atoms per antibody, and this will be the challenge in making the technique work. A variety of polymers that recruit metal ions and bind them with minimal dissociation will be used. This is important because there would be scrambling of the labels if the metal ion complexes were labile. To achieve high sensitivity the polymers must be rather high in molecular weight, which potentially interferes with the recognition by the antibody. This is such a commonly known paradigm that and the recognition sites are known, that it is probably not a show stopper. Using polymers presents another challenge because there is always some level of polydispersity, which can potentially undermine quantitation. Living polymerization is proposed to minimize this problem. Multiple approaches to synthesizing water soluble chelating polymers are proposed, and the immunoassays and ICP-MS are to be accomplished by co-PIs who bring in this expertise. The one weakness in the approach is that the proposal is written as though it is a given that this will work, with the specific aims being simply to create the polymers, tag the antibodies, and run the immunoassays. The proposal does not offer an appreciation for what would be tested at various stages, such as perturbation of the binding constant by the tag, loss of selectivity, and cross-talk.

Innovation: The proposal is highly innovative in proposing a new type of tag that can reasonably achieve a high level of multiplexing.

Investigators: The investigators provide complementary expertise to address this challenging and multifaceted problem.

Environment: The environment is outstanding for carrying out the proposed research.

Overall Evaluation: The proposal offers a very nice and potentially valuable idea that could greatly speed immunoassays. The proposed work is high risk, high impact research. They have a skilled group of investigators and promising preliminary results. The work would benefit from a well thought out strategy for testing the critical issues, including the effect these unusually large tags on binding constants, and possible sacrifices in selectivity from metal ions migrating to other antibodies n the complex mixtures.

CRITIQUE 2:
Significance: The use of ICP-MS is very appropriate to the analysis of biological samples. Detection limits are quite good and are therefore in line with concentrations of biological material isolated in vivo. The ability to provide clinicians and researchers with a better immunoassay that can be used in a multiplex fashion, such as ICP-MS, is very attractive and could have profound ramifications in the medical community.

Approach: The research outlined in this proposal involves the development and implementation of elemental tagging systems together with ICP-MS for providing a better analytical and prognostic tool to clinicians. In particular the PI plans to develop a tagging kit that can be used in immunoassays based on ICP-MS. Most of the developmental work is in the area of organic synthesis and several of the investigators are clearly expert in this area. The use of Ln reagents as metal tags is quite intriguing.

Although the investigators propose to have preliminary data, the data presented is not ICP-MS data. Rather there are fluorescence measurements and ELISA assays that are collected "prior" to the ICPMS analysis.

The organic chemistry proposed in order to make the tagged systems is quite good and the outlined ligands are likely to provide very interesting results providing they can truly complex these systems to antibodies. The investigators propose to tag the primary monoclonal antibodies that are aimed at

epidermal grow factor receptor and have outlined a procedure to do so, although there is some concern as to the expertise of the investigators to do so.

Innovation: The proposed plan is quite innovative as there are very few, if any, others who have conducted the prescribed tagging methods. The use of the Ln ligands is particularly attractive and novel.

Investigators: Dr. Winnik's background is in organic chemistry with a strong background in photochemistry. His particular expertise is in copolymers/polymer chemistry. Dr. Baranov is a physical chemist newly arrived at the U. of Toronto in the biomaterials department. Dr. Nitz has also just begun his career at the U. of Toronto and has a synthetic background as evidenced by his publications with Barbara Imperiali and others. Dr. Ornatsky is the molecular biologist in the group and it will be up to her to transfer much of the proposed synthetic work into a more biological framework. There is some concern that the investigators are heavy in the organic and physical chemistry arena with little experience in the biological aspects necessary to follow through with the proposed research plans.

Environment: The environment appears to be appropriate for the proposed research plan.

Overall Evaluation: This is a very interesting proposal that could in fact have very significant repercussions in the field of clinical prognostic assays if it is successful. Enthusiasm for the planned work would have been much higher had there been better justification of how the PI would meld the organic and physical chemistry aspects with the actual biological endeavor.

CRITIQUE 3:
Few ICP-MS details are given here, although the results in refs 1-5 make it clear ICP-MS can be used to measure metal tags in immunoassays at analyte concentrations of interest. There is no reason to doubt this can be done for many tags at once. The claim that as few as 100 marker molecules could be measured is reasonable if they can tag the antibodies with many metal atoms.

There is a question as to how many analytes can be measured at once by immunoassay due to practical limitations of IA, such as the need to have optimum conditions for binding and washing for many different antigen-antibody reactions, cross-reactivity, nonspecific binding when assays are done for analytes whose concentrations differ over a wide range, etc.

Whether or not 50 analytes can be measured at once, some very interesting and potentially valuable studies are proposed. Dr. Baranov is well-known in ICP-MS as one of the fathers of the dynamic reaction cell for removing polyatomic ion interferences. This has been one of the two or three big new developments in ICP-MS, and therefore in all of analytical atomic spectroscopy, over the last 8 years. Dr. Baranov is also familiar with the techniques needed to handle samples cleanly enough for this work. The ICP-MS instruments and facilities are well-suited to the task. The availability of Drs. Tanner and Bandura will also be helpful. This is the Triumvirate of the DRC. The team members who will work on soluble metal-binding polymers and the biochemical details of the assays are also impressive.

In summary, there is no doubt that ICP-MS under Dr. Baranov will be able to hold up its end of this project. There is significant potential for widely-useful assays to result from this work, and for widespread use of these methods in real clinical environments. ICP-MS instruments no longer require dedicated PhD scientists - they are being run effectively by users with the same sort of training as commonly encountered with clinicians. The studies proposed for metal-binding methods and assay details should lead to good progress in the methodology needed to achieve this goal.

THE FOLLOWING RESUME SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW ADMINISTRATOR TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE ON THE FOLLOWING ISSUES:

COMMITTEE BUDGET RECOMMENDATIONS: The following changes were recommended: Reduction by 1 module ($25,000) per year.

FOREIGN INSTITUTION: This project could add to existing US resources by providing a novel and sensitive clinical assay and is relevant to the advancement of health sciences and to NIH's overall mission.

NOTICE: The NIH has modified its policy regarding the receipt of amended applications. Detailed information can be found by accessing the following URL address: http://grants.nih.gov/grants/policy/amendedapps.htm

NIH announced implementation of Modular Research Grants in the December 18, 1998 issue of the NIH Guide to Grants and Contracts. The main feature of this concept is that grant applications (R01, R03, R21, R15) will request direct costs in $25,000 modules, without budget detail for individual categories. Further information can be obtained from the Modular Grants Web site at http://grants.nih.gov/grants/funding/modular/modular.htm